(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 February 2001 (15.02.2001)

PCT

(10) International Publication Number
WO 01/10882 A1

(51) International Patent Classification[7]: C07H 19/10, 19/20

(21) International Application Number: PCT/CA00/00914

(22) International Filing Date: 10 August 2000 (10.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/147,820 10 August 1999 (10.08.1999) US

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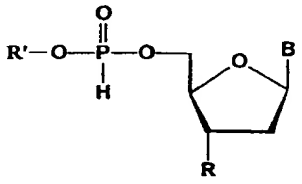
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DEPOT FORMS OF MODIFIED NUCLEOSIDE 5'-HYDROGENPHOSPHATES AS INHIBITORS OF REPRODUCTION OF HUMAN IMMUNODEFICIENCY VIRUS AND HUMAN HEPATITIS B VIRUS



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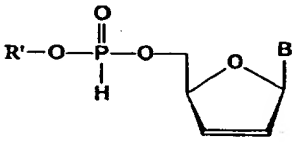

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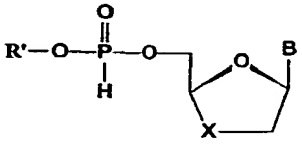

(VIII)

(57) Abstract: The present invention is directed to novel compounds exhibiting a selective inhibition of the reproduction of HIV and HBV and possessing low toxicity. The present compounds are VI-VIII of formulae (VI, VII and VIII) wherein: Nucleosides of D and L-series; B = thymine, adenine, guanine, cytosine, uracyl, 5-fluorouracyl or 5-ethyluracyl; R = N_3, NH_2, F, H or F; X = S or O; R = adamantyl-1, bicyclo[6,5,1] heptyl-1 or *tert.*-butyl. These compounds being depot forms of 5'-hydrogenphosphonates of modified nucleosides are able to inhibit the reproduction of HIV and HBV and are less toxic as compared to the prior art compounds.

WO 01/10882 A1

DEPOT FORMS OF MODIFIED NUCLEOSIDE 5'-HYDROGENPHOSPHATES AS INHIBITORS OF REPRODUCTION OF HUMAN IMMUNODEFICIENCY VIRUS AND HUMAN HEPATITIS B VIRUS

FIELD OF THE INVENTION

The present invention relates to the art of molecular biology and virology. More specifically, in one of its aspects, the present invention relates to novel depot forms of modified 5'-hydrogenphoshonates as selective inhibitors of the reproduction of Human Immunodeficiency Virus (HIV) and Human Hepatitis B Virus (HBV) in cell cultures . In another of its aspects, the present invention relates to a process for producing depot forms of modified 5'-hydrogenphoshonates.

DESCRIPTION OF THE PRIOR ART

It is known in the art that various 5'-hydrogenphosphonates of 3'-azido-3'-deoxythymidine (phosphazide) [1-3] and other modified nucleosides [4-6] inhibit the reproduction of the HIV. While somewhat effective, these compounds are comparatively hydrophilic because of the presence of the unprotected phosphonate group thereby decreasing diffusion thereof into cells.

To the knowledge of the present inventors, only one attempt has been made to prepare a depot form of phosphazide [7], but in human serum these compounds are hydrolyzed to 3'-azido-3'-deoxythymidine rather than to phosphazide. This pathway dominates over the other due to hydrolysis of the nucleoside-phosphonate bond proceeding more rapidly than the elimination of the phosphonate protective group. An example of a depot form of 2',3'-dideoxy-2',3'-didehydrothymidine hydrogenphosphonate has been published [8], but the structure of this compound was not taught.

SUMMARY OF THE INVENTION

We have discovered a principal for the design of depot forms of nucleoside 5'-hydrogenphosphonates and a group of depot forms of modified nucleoside 5'-hydrogenphosphonates having anti HIV and HBV activity. The compounds according to the present invention, viz. depot forms of modified nucleoside 5'-hydrogen-phosphonates, are able to inhibit selectively the reproduction of HIV and HBV in cell cultures.

DEPOT FORMS OF MODIFIED NUCLEOSIDE 5'-HYDROGENPHOSPHATES AS INHIBITORS OF REPRODUCTION OF HUMAN IMMUNODEFICIENCY VIRUS AND HUMAN HEPATITIS B VIRUS

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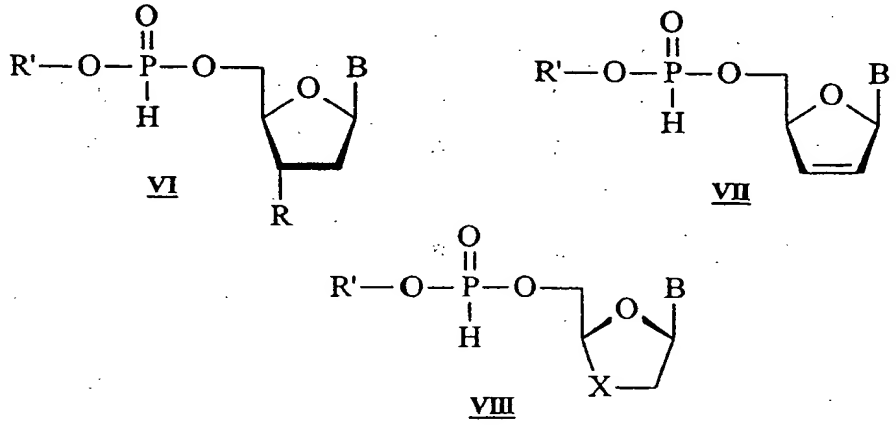
We have discovered a principal for the design of depot forms of nucleoside 5'-hydrogenphosphonates and a group of depot forms of modified nucleoside 5'-hydrogenphosphonates having anti HIV and HBV activity. The compounds according to the present invention, viz. depot forms of modified nucleoside 5'-hydrogen-phosphonates, are able to inhibit selectively the reproduction of HIV and HBV in cell cultures.

According to the synthetic pathway set out in Figure 1, prodrug **I** can be hydrolyzed in two possible ways: to nucleoside 5′-hydrogenphosphonate (**II**, Route **a**) or nucleoside **III** (Route **b**). The route of choice, inter alia, depends on the stability and steric availability of the corresponding bonds in **I**. Our study of several groups of prodrugs in human serum showed that most compounds were hydrolyzed via Route **b** except for those bearing tertiary alkyl esters, which were hydrolyzed via Route **a**.

The third possibility is the preliminary oxidation of **I** to **IV** via Route **c** with subsequent hydrolysis either to **V** (via Route **d**) or to **III** (via Route **e**). It is known that oxidation proceeds much faster for hydrogenphosphonate diesters than for hydrogenphosphonate monoesters.

The study of diesters of nucleoside hydrogenphosphonates in human serum showed that most compounds were hydrolyzed via Route **b** except for those bearing tertiary alkyl esters, which were hydrolyzed via Route **a**.

Prodrug forms of 5'-hydrogenphosphonates of modified nucleosides hydrolyzed to the corresponding nucleoside 5′-hydrogenphosphonates include:



VI

VII

VIII

wherein:

nucleosides of D and L-series,;

B = thymine, adenine, guanine, cytosine, uracyl, 5-fluorouracyl or 5-ethyluracyl;

R = N_3, H or F;

X = S or O; and

R' = adamantyl-1, bicyclo[2,2,1]heptyl-1, *tert.*-butyl.

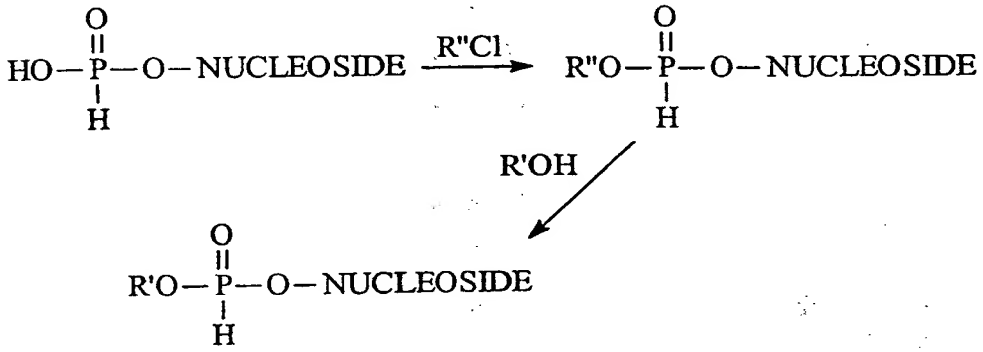
BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the present invention will be described with reference to the accompanying drawing, in which:

Figure 1 illustrates a synthetic pathway, inter alia, for the production of prodrug I.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The synthesis of **VI-VIII** with thymine and adenine bases may be achieved according to the following reaction scheme (referred to hereinbelow as Scheme 2):



wherein:

NUCLEOSIDE = nucleoside component;

R'- = adamantyl-1, bicyclo[2,2,1] heptyl-1 or *tert.*-butyl; and

R'' - $COC(CH_3)_3$, $-SO_2CH_3$ or $-SO_2CF_3$.

The synthesis of **VI-VIII** with thymine, adenine, and guanine bases may be achieved according to the following reaction scheme (referred to hereinbelow as Scheme 3):

R' = adamantyl-1, bicyclo[2,2,1]heptyl-1, *tert.*-butyl.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the present invention will be described with reference to the accompanying drawing, in which:

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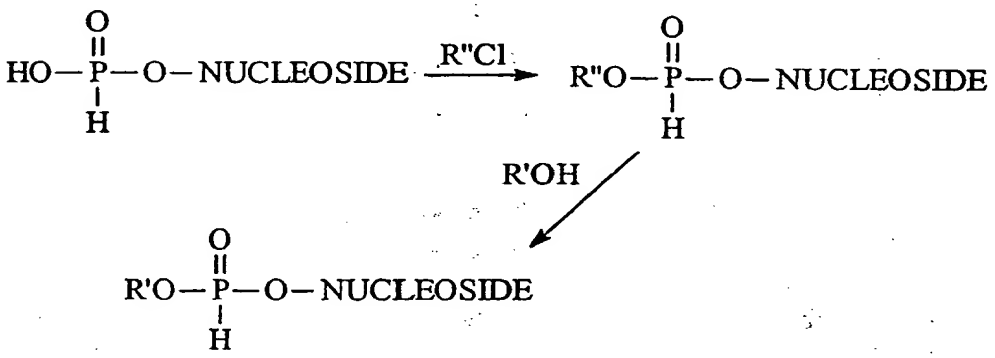

wherein:

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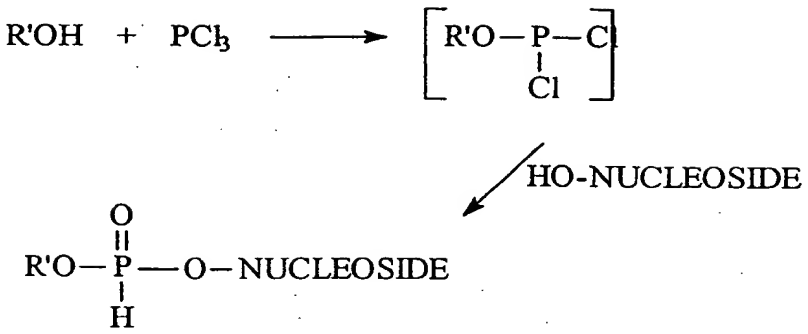
The synthesis of **VI-VIII** with thymine, adenine, and guanine bases may be achieved according to the following reaction scheme (referred to hereinbelow as Scheme 3):

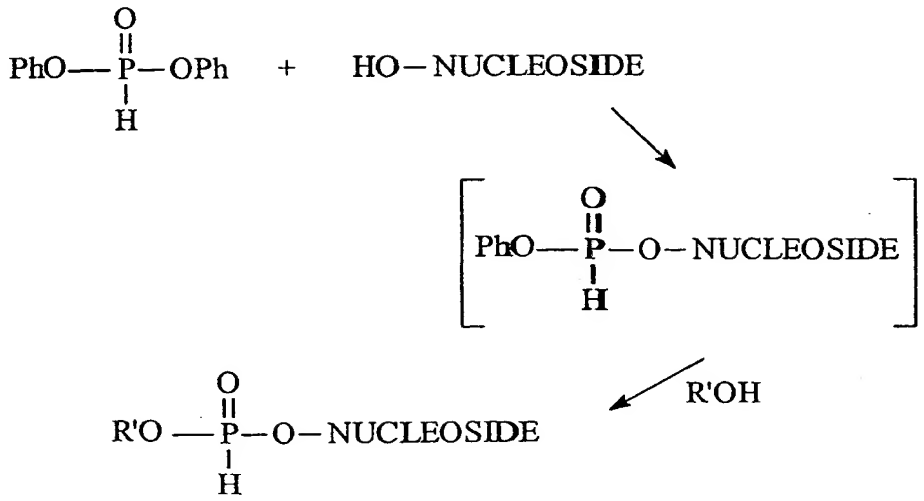


wherein:

NUCLEOSIDE = nucleoside component; and

R′ = adamantyl-1, bicyclo[2,2,1]heptyl-1 or *tert.*-butyl.

The synthesis of **VI-VIII** with different nucleic bases may be achieved according the following reaction scheme (referred to hereinbelow as Scheme 4):



wherein:

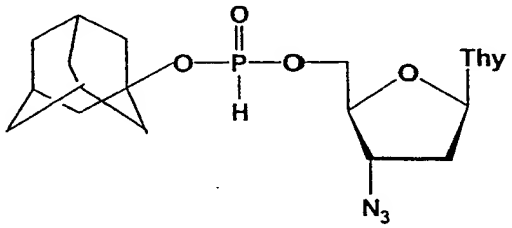
NUCLEOSIDE = nucleoside component;

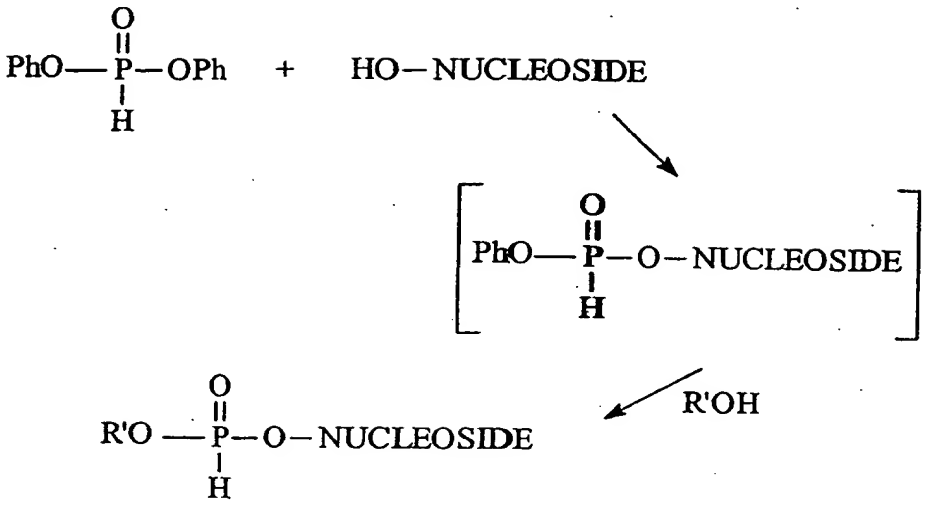
R' = adamantyl-1, bicyclo[2,2,1] heptyl-1 or *tert.*-butyl.

Embodiments of the present invention will be described with reference to the following Examples which are provided for illustrative purposes only and should not be use to construe or limited the scope of the present invention.

[A] EXAMPLES OF THE SYNTHETIC PROCEDURE

EXAMPLE 1: P-(Adamantyl-1) 3′-azido-2′,3′-dideoxythymidine 5'-hydrogenphosphonate (**VIa**, B=Thy, R=N_3, R`=adamantyl-1) - Scheme 2





wherein:

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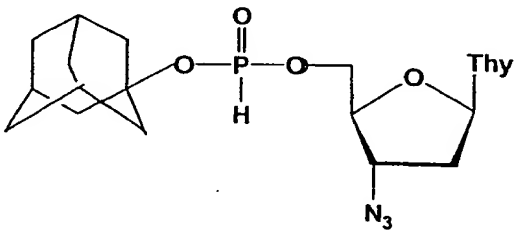
[A] EXAMPLES OF THE SYNTHETIC PROCEDURE

EXAMPLE 1: *P*-(Adamantyl-1) 3′-azido-2′,3′-dideoxythymidine 5'-hydrogenphosphonate (VIa, B=Thy, R=N_3, R`=adamantyl-1) - Scheme 2

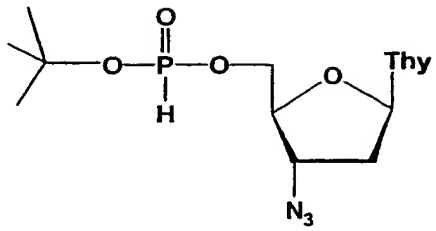


A water solution (0.5 ml) of 3′-azido-2′,3′-dideoxythymidine 5′-hydrogenphosphonate (Phosphazide) Na-salt (88 mg, 0.25 mmol) was passed through a Dowex-50 (Py^+) column (5 x 1 cm), the column washed with water until no UV-adsorption at 260 nm was observed. The eluate was evaporated to dryness and coevaporated with pyridine (3 x 2 ml). The residue was dissolved in MeCN (5 ml), cooled to -20°C and adamantanol-1 (58 mg, 0.4 mmol) was added under stirring. Pyridine (0.5 ml) and pivaloyl chloride (90 mg, 91 ml, 0.75 mmol) were added, cooling was removed and the reaction mixture was stirred for 10 min. The mixture was diluted with chloroform (10 ml) and washed with cooled saturated sodium bicarbonate (5 ml) and water (3 x 3 ml). The organic solution was dried with Na_2SO_4, evaporated, and reevaporated with toluene. The product was purified on a Kieselgel 60 column (15 x 2.5 cm) eluting with chloroform : methanol 95 : 5. The target fractions were evaporated to give compound **VIa**. The yield and physicochemical data are given in Tables 1-3.

Example 2: *P*-(Adamantyl-1) 3′-azido-2′,3′-dideoxythymidine 5'-hydrogenphosphonate (**VIa**, B=Thy, R=N_3, R`=adamantyl-1) - Scheme 3

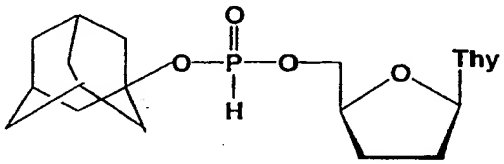
A solution of PCl_3 (75 mg, 44 ml, 0.5 mmol) in dichloromethane (2 ml) was cooled to 1-2°C, adamantanol-1 (79 mg, 0.5 mmol) and pyridine (40 ml, 0.5 mmol) were added for 20 min at 4-5°C under stirring. A solution of 3′-azido-2′,3′-dideoxythymidine prepared from Na-salt (65 mg, 0.25 mmol) as in the example 1 in pyridine (2 ml) was dropped for 3 h. The reaction mixture was stirred for 3 h at 18°C and then quenched with chloroform (5 ml) and a cooled solution of 1.5 M NH_4HCO_3 (3 ml), the organic layer was washed with water, dried with sodium sulfate, and evaporated. The residue was purified on a Kieselgel 60 column (2 x 25 mm) eluting with $CHCl_3$-*tert.*-BuOH 9 : 1 to give compound **VIa**. The yield and physicochemical data are given in Tables 1-3.

Example 3: *P-tert.*-Butyl 3′-azido-2′,3′-dideoxythymidine 5'-hydrogenphosphonate (**VIb**, B=Thy, R=N_3, R`=*tert.*-butyl) - Scheme 2



Phosphazide Na-salt (88 mg, 0.25 mmol) was coupled with *tert.*-butanol (24 mg, 35 ml, 0.38 mmol) under the conditions of example 1. For the yield and physicochemical data, see Tables 1-3.

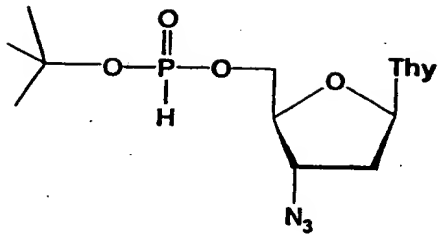
Example 4: *P*-(Adamantyl-1) 2′,3′-dideoxythymidine 5′-hydrogenphosphonate (**VIc**, B=Thy, R=H, R`=adamantyl-1) - Scheme 2.



2′,3′-Dideoxythymidine 5′-hydrogenphosphonate Na-salt (78 mg, 0.25 mmol) was coupled with adamantanol-1 (58 mg, 0.4 mmol) under the conditions of Example 1 to give **VIc**. For the yield and physicochemical data, see Tables 1-3.

Example 5: *P*-(Adamantyl-1) 2′,3′-dideoxythymidine 5′-hydrogenphosphonate (**VIc**, B=Thy, R=H, R`=adamantyl-1) - Scheme 4.

A solution of 2′,3′-dideoxythymidine 5′-hydrogenphosphonate (65 mg, 0.25 mmol) in dichloromethane (1 ml) and pyridine (1 ml) was added to the solution of diphenylphosphite (97 mg, 65 ml, 0.5 mmol) in acetonitrile (2 ml) at 0°C and, after 30-min stirring, adamantanol-1 (58 mg, 0.4 mmol) was added to the reaction mixture under stirring. After 1 h, the mixture was diluted with triethylamine - water 1 : 1, evaporated,



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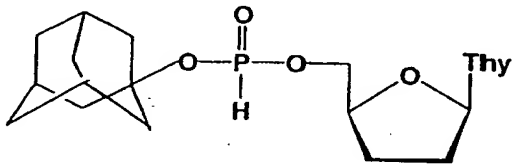


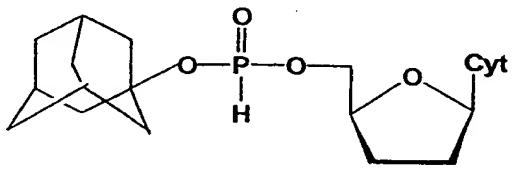
2′,3′-Dideoxythymidine 5′-hydrogenphosphonate Na-salt (78 mg, 0.25 mmol) was coupled with adamantanol-1 (58 mg, 0.4 mmol) under the conditions of Example 1 to give **VIc**. For the yield and physicochemical data, see Tables 1-3.

Example 5: *P*-(Adamantyl-1)2′,3′-dideoxythymidine 5′-hydrogenphosphonate (**VIc**, B=Thy, R=H, R`=adamantyl-1) - Scheme 4.

A solution of 2′,3′-dideoxythymidine 5′-hydrogenphosphonate (65 mg, 0.25 mmol) in dichloromethane (1 ml) and pyridine (1 ml) was added to the solution of diphenylphosphite (97 mg, 65 ml, 0.5 mmol) in acetonitrile (2 ml) at 0°C and, after 30-min stirring, adamantanol-1 (58 mg, 0.4 mmol) was added to the reaction mixture under stirring. After 1 h, the mixture was diluted with triethylamine - water 1 : 1, evaporated,

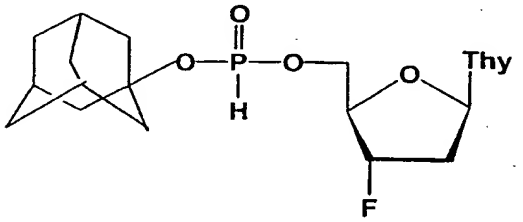
the residue was diluted with chloroform (3 ml), washed with water (3 x 1 ml), and the residue chromatographed on a Kieselgel 60 column (2 x 25 mm) eluting with $CHCl_3$-ethanol 9 : 1 to give compound **VIc**. The yield and physicochemical data are given in Tables 1-3.

Example 6: *P*-(Adamantyl-1) 2′,3′-dideoxycytidine 5′-hydrogenphosphonate (**VId**, B=Cyt, R=H, R`=adamantyl-1). Scheme 4



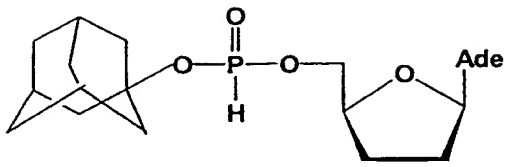
2′,3′-Dideoxycytidine (105 mg, 0.5 mmol), adamantanol-1 (116 mg, 0.8 mmol), and diphenylphosphite (194 mg, 130 ml, 1 mmol) were coupled under the conditions of example 5. For the yield and physocochemical data of **VId**, see Tables 1-3.

Example 7: *P*-(Adamantyl-1) 3′-fluoro-2′,3′-dideoxythymidine 5′-hydrogenphosphonate (**VIe**, B=Thy, R=F, R`=adamantyl-1) - Scheme 2



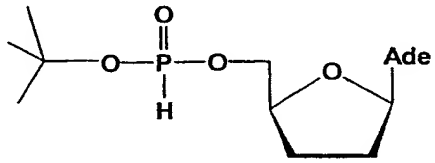
3′-Fluoro-2′,3′-dideoxythymidine 5′-hydrogenphosphonate Na-salt (82 mg, 0.25 mmol) was converted to **VIe** as described in example 1. The yield and physicochemical data are given in Tables 1-3.

Example 8: *P*-(Adamantyl-1) 2′,3′-dideoxyadenosine 5′-hydrogenphosphonate (**VIf**, B=Ade, R=H, R`=Adamantyl-1) - Scheme 4



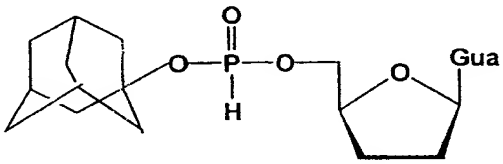
2′,3′-Dideoxyadenosine (118 mg, 0.5 mmol) was converted to **VIf** under the conditions of example 6. The yield and physicochemical data of **VIf** are given in Tables 1-3.

Example 9: *P-tert.*-Butyl 2′,3′-dideoxyadenosine 5′-hydrogenphosphonate (**VIg**, B=Ade, R=H, R`=*tert.*-butyl) - Scheme 4.



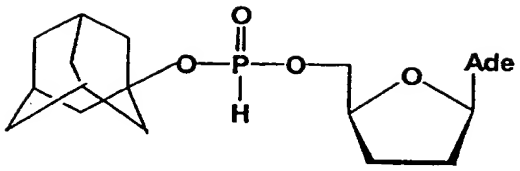
2′,3′-Dideoxyadenosine (118 mg, 0.5 mmol) was converted to **VIg** under the conditions of example 6. The yield and physicochemical data are given in Tables 1-3.

Example 10: *P*-(Adamantyl-1) 2′,3′-dideoxyguanosine 5′-hydrogenphosphonate (**VIh**, B=Gua, R=H, R`=adamantyl-1) - Scheme 4



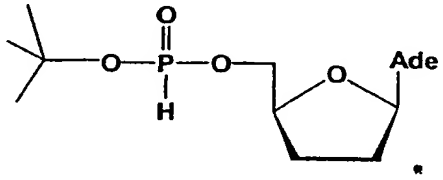
2′,3′-Dideoxyguanosine (125 mg, 0.5 mmol) was converted to **VIh**. as in the Example 6. The yield and physicochemical data are given in Tables 1-3.

Example 11: *P*-(Adamantyl-1) 2′,3′-dideoxy-2′,3′-didehydrothymidine 5′-hydrogenphosphonate (**VIIa**, B=Thy, R`=adamantyl-1) - Scheme 2



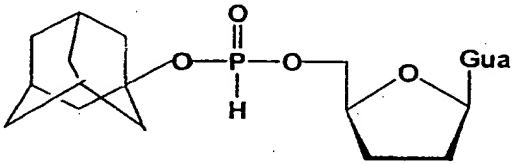
2′,3′-Dideoxyadenosine (118 mg, 0.5 mmol) was converted to **VIf** under the conditions of example 6. The yield and physicochemical data of **VIf** are given in Tables 1-3.

Example 9: *P-tert.*-Butyl 2′,3′-dideoxyadenosine 5′-hydrogenphosphonate (**VIg**, B=Ade, R=H, R`=*tert.*-butyl) - Scheme 4.



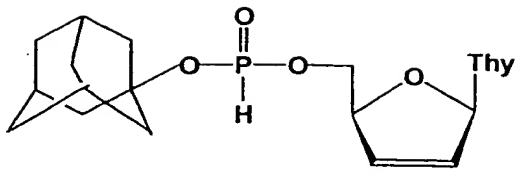
2′,3′-Dideoxyadenosine (118 mg, 0.5 mmol) was converted to **VIg** under the conditions of example 6. The yield and physicochemical data are given in Tables 1-3.

Example 10: *P*-(Adamantyl-1) 2′,3′-dideoxyguanosine 5′-hydrogenphosphonate (**VIh**, B=Gua, R=H, R`=adamantyl-1) - Scheme 4



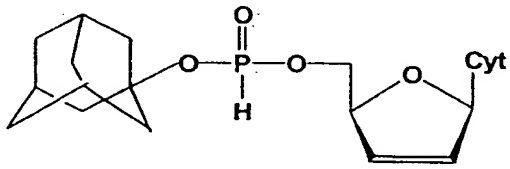
2′,3′-Dideoxyguanosine (125 mg, 0.5 mmol) was converted to **VIh**. as in the **Example** 6. The yield and physicochemical data are given in Tables 1-3.

Example 11: *P*-(Adamantyl-1) 2′,3′-dideoxy-2′,3′-didehydrothymidine 5′-hydrogenphosphonate (**VIIa**, B=Thy, R`=adamantyl-1) - Scheme 2



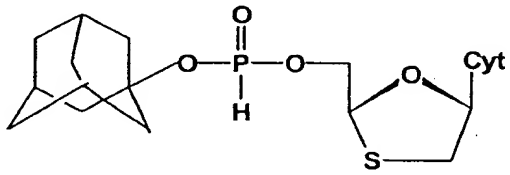
2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-hydrogenphosphonate Na-salt (78 mg, 0.25 mmol) was converted to **VIIa** under the conditions of example 1. The yield and physicochemical data are given in Tables 1-3.

Example 12: *P*-(Adamantyl-1) 2′,3′-dideoxy-2′,3′-didehydrocytidine 5′-hydrogenphosphonate (**VIIb** B=Cyt, R`=adamantyl-1) - Scheme 4



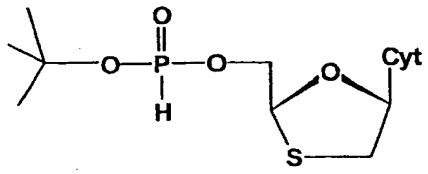
VIIb was synthesized from 2′,3′-dideoxy-2′,3′-didehydrocytidine (125 mg, 0.5 mmol) under the conditions of Example 6. The yield and physicochemical data are given in Tables 1-3.

Example 13: *P*-(Adamantyl-1) L-3′-thiocytidine 5′-hydrogenphosphonate (**VIIIa**, B=Cyt, R`=adamantyl-1) - Scheme 3



Compound **VIIIa** was synthesized from L-3′-thiocytidine (120 mg, 0.5 mmol) under the conditions of example 3. The yield and physicochemical data are given in Tables 1-3.

Example 14: *P-tert.*-Butyl L-3′-thiocytidine 5′-hydrogenphosphonate (**VIIIb**, B=Cyt, R`=*tert.*-butyl) - Scheme 4



Compound **VIIIb** was synthesized from L-3′-thiocytidine (120 mg, 0.5 mmol) according to Example 3. The yield and physicochemical data are given in Tables 1-3.

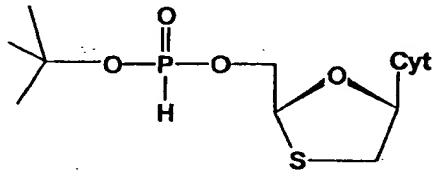
TABLE 1

Yields and physicochemical characteristics

Compound	R	Mol. mass	Yield, %	UV, MeOH, λ_{max}, nm
VIa	adamantyl-1	463	67	264
VIb	*tert.*-butyl	387	76	265
VIc	adamantyl-1	421	78	264
VId	adamantyl-1	407	61	274
VIe	adamantyl-1	439	88	265
VIf	adamantyl-1	431	65	257
VIg	*tert.*-butyl	355	59	257
VIh	adamantyl-1	447	59	250
VIIa	adamantyl-1	419	79	265
VIIb	adamantyl-1	415	62	274
VIIIa	adamantyl-1	425	57	272
VIIIb	*tert.*-butyl	349	48	273

Compound **VIIIa** was synthesized from L-3′-thiocytidine (120 mg, 0.5 mmol) under the conditions of example 3. The yield and physicochemical data are given in Tables 1-3.

Example 14: *P-tert.*-Butyl L-3′-thiocytidine 5′-hydrogenphosphonate (**VIIIb**, B=Cyt, R`=*tert.*-butyl) - Scheme 4



Compound **VIIIb** was synthesized from L-3′-thiocytidine (120 mg, 0.5 mmol) according to Example 3. The yield and physicochemical data are given in Tables 1-3.

TABLE 1

Yields and physicochemical characteristics

Compound	R	Mol. mass	Yield, %	UV, MeOH, λ_{max}, nm
VIa	adamantyl-1	463	67	264
VIb	*tert.*-butyl	387	76	265
VIc	adamantyl-1	421	78	264
VId	adamantyl-1	407	61	274
VIe	adamantyl-1	439	88	265
VIf	adamantyl-1	431	65	257
VIg	*tert.*-butyl	355	59	257
VIh	adamantyl-1	447	59	250
VIIa	adamantyl-1	419	79	265
VIIb	adamantyl-1	415	62	274
VIIIa	adamantyl-1	425	57	272
VIIIb	*tert.*-butyl	349	48	273

TABLE 2.

^{1}H NMR spectra of VI-VIII, 200 MHz; CD_3CN; δ, ppm; (J, Hz):

Comp.	H1' 1H	H2'	H3' 1H	H4' 1H	H5' 2H	H-5 (pyr) or H-2 (pur) 1H	H-6 (pyr) or H-8 (pur)1H	CH_3, Thy	P^{31}
VIa	6.14dd (7)	2.38m 2H	4.34m	4.01m	4.15m	-	7.38 q (1)	1.96d (7)	6.93d (696)
VIb	6.15dd (6.5)	2.38m, 2H	4.37m	4.01m	4.20m	-	7.38q (1)	1.8d (6.5)	6.90d (697)
VIc	6.05m	4.4m, 2H	5.03t (10.7)	4.2m	3.62q (6.55)	-	6.45d (7.3)	1.7d (7.7)	6.48d (638)
VId	6.4m	2.15m, 2H	3.52d (10.7)	4.2m	3.99m	6.35d (7.7)	8.04d (7.7)	-	6.71d (630)
VIe	6.21t (6.6)	2.46t (6.2), 2H	4.63t (5.43)	3.38s	4.2m	-	7.65d (0.5)	1.8d (0.8)	7.11d (638)
VIf	6.34q (7)	2.14m, 2H	2.66m	4.24m	3.56m	8.39s	8.16s	-	6.59d (638)
VIg	6.28q (6.5)	2.26t (6.4), 2H	2.76m	4.28m	3.48m	8.46s	8.04s	-	6.66d (641)
VIh	6.04q (6)	2.12m, 2H	2.49m	4.1m	3.9m	8.00c	-	-	6.56d (632)
VIIa	6.95t (5)	4.98m, 1H	5.25m	4.05m	3.65m	6.45d (7.3)	7.72d (7.3)	-	6.48d (638)
VIIb	6.95t (5.2)	5.12m, 1H	5.31m	4.11m	3.55m	6.35d (7.6)	8.04d (7.7)	-	6.71d (630)
VIIIa	6.6dd (3.0,5.9)	3.4dd (3.0; 5.7),2H	-	5.24t (4.6)	3.65m, 2H	5.88d (7.7)	7.89d (7.7)	-	6.71d (630
VIIIb	6.52dd (3.1.5.8)	3.48dd (3.0; 5.6), 2H	-	5.24t (4.6)	3.72m, 2H	5.80d (7.7)	7.75d (7.7)	-	6.71d (630)

TABLE 3

^{1}H NMR spectral data for "depot" groups in **VI-VIII**, 200 MHz; CD_3CN; δ, ppm; (J, Hz) and ^{31}P NMR spectra of **VI-VIII**, 81 MHz, CD_3CN; δ, ppm, (J, Hz)

Comp.	*tert.*-CH (adam.), 3H	CH_2-C-O- P(adam.), 6H	CH_2(adam.) 6H	*tert.*-Bu, 9H	^{31}P NMR, 1P
VIa	2.16 br.s	2.06m	1.64m	-	3.36d (696), 3.08d
VIb	-	-	-	1.49s	3.99dt (697 and 8), 3.63 dt.
VIc	2.16 br.s	2.06m	1.63m		3.38d (694), 3.04d
VId	2.16 br.s	2.08m	1.64m		3.33d (696), 3.07d
VIe	2.16 br.s	2.03m	1.64m		3.36d (694), 3.06d
VIf	2.16 br.s	2.06m	1.63m		3.32d (695), 3.11d
VIg	-	-	-	1.55s	3.99dt (697 and 8), 3.63 dt.
VIh	2.16 br.s	2.03m	1.66m		3.32d (698), 3.01d
VIIa	2.16 br.s	2.03m	1.65m		3.36d (696), 3.10d
VIIb	2.16 br.s	2.10m	1.67m		3.37d (695), 3.08d
VIIIa	2.16 br.s	2.09m	1.67m		3.35d (694), 3.07d
VIIIb	-	-	-	1.52s	3.94dt (692 and 8), 3.61 dt.

[B] STABILITY IN THE HUMAN SERUM

The compounds according to the present invention are slowly hydrolyzed to corresponding nucleoside 5`-hydrogenphosphonates in human blood serum.

The hydrolysis of the compounds according to the present invention was performed in human blood serum. The assay mixture containing 0.5 ml of 100 μM solution of the compound of the invention in formamide and 99.5 μl of 100% human fetal serum was incubated at 37°C. After certain intervals, the 5-μl samples were mixed with 45 μl of 10% trifluoroacetic acid, centrifuged for 10 min at 12,000 rpm, and the supernatants were concentrated to 100 μl and analyzed by HPLC on a Silasorb C7 column (4 x 150 mm, 13 m) with a linear gradient of methanol from 0 to 80% in 0.05 M potassium phosphate buffer (pH 6.0) for 40 min. The flow rate was 0.5 ml/min. The extent of hydrolysis was assessed by measuring the amount of the product.

The results of the tests are shown in Table 4 hereinbelow.

TABLE 3

^{1}H NMR spectral data for "depot" groups in VI-VIII, 200 MHz; CD_3CN; δ, ppm; (J, Hz) and ^{31}P NMR spectra of VI-VIII, 81 MHz, CD_3CN; δ, ppm, (J, Hz)

Comp.	*tert.*-CH (adam.), 3H	CH_2-C-O-P(adam.), 6H	CH_2(adam.) 6H	*tert.*-Bu, 9H	^{31}P NMR, 1P
VIa	2.16 br.s	2.06m	1.64m	-	3.36d (696), 3.08d
VIb	-	-	-	1.49s	3.99dt (697 and 8), 3.63 dt.
VIc	2.16 br.s	2.06m	1.63m		3.38d (694), 3.04d
VId	2.16 br.s	2.08m	1.64m		3.33d (696), 3.07d
VIe	2.16 br.s	2.08m	1.64m		3.36d (694), 3.06d
VIf	2.16 br.s	2.06m	1.63m		3.32d (695), 3.11d
VIg	-	-	-	1.55s	3.99dt (697 and 8), 3.63 dt.
VIh	2.16 br.s	2.03m	1.66m		3.32d (698), 3.01d
VIIa	2.16 br.s	2.03m	1.65m		3.36d (696), 3.10d
VIIb	2.16 br.s	2.10m	1.67m		3.37d (695), 3.08d
VIIIa	2.16 br.s	2.09m	1.67m		3.35d (694), 3.07d
VIIIb	-	-	-	1.52s	3.94dt (692 and 8), 3.61 dt.

[B] STABILITY IN THE HUMAN SERUM

The compounds according to the present invention are slowly hydrolyzed to corresponding nucleoside 5`-hydrogenphosphonates in human blood serum.

The hydrolysis of the compounds according to the present invention was performed in human blood serum. The assay mixture containing 0.5 ml of 100 μM solution of the compound of the invention in formamide and 99.5 μl of 100% human fetal serum was incubated at 37°C. After certain intervals, the 5-μl samples were mixed with 45 μl of 10% trifluoroacetic acid, centrifuged for 10 min at 12,000 rpm, and the supernatants were concentrated to 100 μl and analyzed by HPLC on a Silasorb C7 column (4 x 150 mm, 13 m) with a linear gradient of methanol from 0 to 80% in 0.05 M potassium phosphate buffer (pH 6.0) for 40 min. The flow rate was 0.5 ml/min. The extent of hydrolysis was assessed by measuring the amount of the product.

The results of the tests are shown in Table 4 hereinbelow.

TABLE 4

Hydrolysis of 50% of the compounds under the invention to corresponding nucleoside 5′-phosphonates

Compound	Time, min
VIa	60
VIb	5
VIc	55
VId	35
VIe	42
VIf	85
VIg	79
VIh	66
VIIa	150
VIIb	18
VIIIa	75
VIIIb	20

As seen in Table 4, in most cases the half-lives of the compounds according to the present invention are rather high.

TABLE 5

Concentration of the compounds under the invention inhibiting the HIV reproduction

Compounds of the invention	Concentration, inhibiting the virus reproduction by 50%, nM	concentration inhibiting the virus reproduction by 95%, nM	Concentration inhibiting cell growth for 50%, μM
VIa	12	50	2800
VIb	45	210	3100
VIc	35	180	6800
VId	5	65	120
VIe	0.8	6	2400
VIf	14	95	5450
VIg	32	430	4300
VIh	88	590	4460
VIIa	88	460	3600
VIIb	56	235	2900
VIIc	92	430	280
VIIIa	33	160	890
VIIIb	37	165	165
AZT	3	16	1800
ddT	2500		>10000
ddC	2	14	80
FLT	2	8	1400
ddA	150	720	3000
ddG	400	1260	2480
d4T	80	310	2400
d4C	120	465	120
3TC	65	140	860

AZT - 3'-azido-2',3'-dideoxythymidine, FLT - 3'-fluoro-2',3'-dideoxythymidine

The data presented in Table 5 demonstrate that antiviral activity of the compounds according to the present invention is comparable to that of AZT and other nucleosides and is sometimes higher.

[C] ANTI-HIV ACTIVITY OF THE INVENTED COMPOUNDS

The data presented in Table 5 demonstrate antiviral activity and toxicity in HIV-infected MT4 cell culture of the compounds according to the present invention.

Antiviral activity and toxicity were studied according to [4,6].

TABLE 5

Concentration of the compounds under the invention inhibiting the HIV reproduction

Compounds of the invention	Concentration, inhibiting the virus reproduction by 50%, nM	concentration inhibiting the virus reproduction by 95%, nM	Concentration inhibiting cell growth for 50%, μM
VIa	12	50	2800
VIb	45	210	3100
VIc	35	180	6800
VId	5	65	120
VIe	0.8	6	2400
VIf	14	95	5450
VIg	32	430	4300
VIh	88	590	4460
VIIa	88	460	3600
VIIb	56	235	2900
VIIc	92	430	280
VIIIa	33	160	890
VIIIb	37	165	165
AZT	3	16	1800
ddT	2500		>10000
ddC	2	14	80
FLT	2	8	1400
ddA	150	720	3000
ddG	400	1260	2480
d4T	80	310	2400
d4C	120	465	120
3TC	65	140	860

AZT - 3'-azido-2',3'-dideoxythymidine, FLT - 3'-fluoro-2',3'-dideoxythymidine

The data presented in Table 5 demonstrate that antiviral activity of the compounds according to the present invention is comparable to that of AZT and other nucleosides and is sometimes higher.

[C] ANTI-HIV ACTIVITY OF THE INVENTED COMPOUNDS

The data presented in Table 5 demonstrate antiviral activity and toxicity in HIV-infected MT4 cell culture of the compounds according to the present invention.

Antiviral activity and toxicity were studied according to [4,6].

REFERENCES

The following publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety:

1. Tarussova N.B., Khorlin A.A., Krayevsky A.A., Korneyeva M.N., Nosik D.N., Kruglov I.V., Galegov G.A., Beabealashvilli R.Sh., Inhibition of human immunodefifciency virus in cell culture by 3'-azydo-2',3'-dideoxynucleosides, *Ìîl.Biol. Russian,* 1989, **23**, N6, 1716-1724.

2. Tarussova N.B., Kukhanova M.K., Krayevsky A.A., Karamov E.V., Lukashov V.V., Kornilayeva G.V., Rodina M.A., Galegov G.A., Inhibition of human immunodeficiency virus (HIV) production by 5'-hydrogenphospho-nates of 3'-azido-2',3'-deoxynucleosides, *Nucleosides & Nucleotides*, 1991, **10**, N1-3, 351-354.

3. Khorlin A.A., Tarusova N.B., Dyatkina N.B., Krayevsky A.A., Beabealashvilli R.Sh., Galegov G.A., Zhdanov V.M., Korneeva M.S., Nosik D.N., Maiorova S.N., Shobukhov V.M., **Patent RF 1 548 182**, 16.02.1992, **US Patent 5,043,437**, 27.8.1991; **European Patent 0-354-246**, 16.03.1994; **Japan Patent N 0-354-246 B1**, 08.25.1995; **Corean Patent 106,957**, 12.01.96.

4. Atrazheva E.D., Lukin M.A., Jasko M.V., Shushkova T.V., Tarussova N.B., Krayevsky A.A., Balzarini J., De Clercq E., 2',3'-O-Cyclic derivatives of ribonucleosides and their 5'-phosphonates: synthesis and anti-HIV activity, *Med.Chem.Res.*, 1991, **1**, 155-165.

5. Karamov E.V., Lukashov V.V., Gorbachova A.P., Kornilayeva G.V., Tarussova N.B., Krayevsky A.A., Inhibition of HIV reproduction in cell cultures by 2',3'-dideoxynucleoside 5'-phosphites, *Ìîl.Biol. Russian*, 1992, **26**, N1, 201-207).

6. Krayevsky A.A., Tarussova N.B., Zhu Q.-Y., Vidal P., Chou T.-C., Baron P., Polsky B., Jiang X.-Y., Matulic-Adamic J., Rosenberg I., Watanabe K.A., 5'-Hydrogenphosphonates and 5'-methylphosphonates of sugar modified pyrimidine nucleosides as potential anti-HIV agents. *Nucleosides & Nucleotides*, 1992, **11**, N2-4, 177-196.

7. Mc Guigan C., Bellevergue P., Jones B.C.N.M., Mahmood N., Hay A.J., Petrik J., Karpas A., Alkyl hydrogen phosphonate phosphonate derivatives of anti-HIV agent AZT may be less toxic than parent nucleosides, *Antiviral Chem., Chemother.*, 1994, **5**, N4, 271-277.

8. Cardona V.M.F., Ayi A.L., Aubertin A.M., Guedj R., Anti-HIV activity of new compounds: Prodrug of D4T, Antiviral Res., 1998, 37, N3 A52.

6. Krayevsky A.A., Tarussova N.B., Zhu Q.-Y., Vidal P., Chou T.-C., Baron P., Polsky B., Jiang X.-Y., Matulic-Adamic J., Rosenberg I., Watanabe K.A., 5'-Hydrogenphosphonates and 5'-methylphosphonates of sugar modified pyrimidine nucleosides as potential anti-HIV agents. *Nucleosides & Nucleotides*, 1992, 11, N2-4, 177-196.

7. Mc Guigan C., Bellevergue P., Jones B.C.N.M., Mahmood N., Hay A.J., Petrik J., Karpas A., Alkyl hydrogen phosphonate phosphonate derivatives of anti-HIV agent AZT may be less toxic than parent nucleosides, *Antiviral Chem., Chemother.*, 1994, 5, N4, 271-277.

8. Cardona V.M.F., Ayi A.L., Aubertin A.M., Guedj R., Anti-HIV activity of new compounds: Prodrug of D4T, Antiviral Res., 1998, 37, N3 A52.

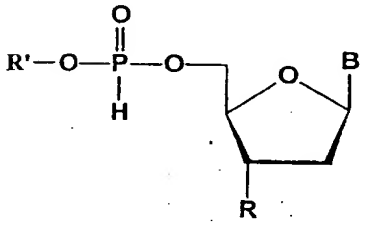
What is claimed is:

1. Adamantyl esters of 5′-O-hydrogenphosphonates of 3′-azido-2′,3′-dideoxythymidine (phoshazide), 2′,3′-dideoxythymidine, 3′-amino-2′,3′-dideoxythymidine, 3′-fluoro-2′,3′-dideoxythymidine, 2′,3′-dideoxy-2′,3′-didehydrothymidine, 2′,3′-dideoxycytidine, 2′,3′-dideoxyadenosine, 2′,3′-dideoxyguanosine, L-3′-thiocytidine.

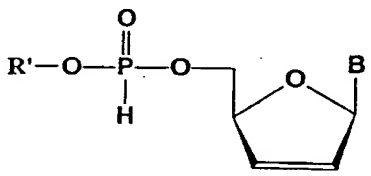
2. *tert.*-Butyl esters of 5′-O-hydrogenphosphonates of 3′-azido-2′,3′-dideoxythymidine (phoshazide), 2′,3′-dideoxythymidine, 3′-amino-2′,3′-dideoxythymidine, 3′-fluoro-2′,3′-dideoxythymidine, 2′,3′-dideoxy-2′,3′-didehydrothymidine, 2′,3′-dideoxycytidine, 2′,3′-dideoxyadenosine, 2′,3′-dideoxyguanosine, L-3′-thiocytidine.

3. Bicyclo[2,2,1] heptyl esters of 5′-O-hydrogenphosphonates of 3′-azido-2′,3′-dideoxythymidine (phoshazide), 2′,3′-dideoxythymidine, 3′-amino-2′,3′-dideoxythymidine, 3′-fluoro-2′,3′-dideoxythymidine, 2′,3′-dideoxy-2′,3′-didehydrothymidine, 2′,3′-dideoxycytidine, 2′,3′-dideoxyadenosine, 2′,3′-dideoxyguanosine, L-3′-thiocytidine..

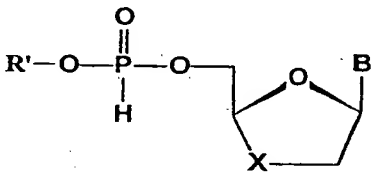
4. Compounds of the formulae **VI-VIII**:



VI



VII



VIII

wherein:

Nucleosides of D and L-series;

B = thymine, adenine, guanine, cytosine, uracyl, 5-fluorouracyl or 5-ethyluracyl;

R = N_3, NH_2, F, H or F;

X = S or O;

R = adamantyl-1, bicyclo[6,5,1] heptyl-1 or *tert.*-butyl.

B = thymine, adenine, guanine, cytosine, uracyl, 5-fluorouracyl or 5-ethyluracyl;

R = N_3, NH_2, F, H or F;

X = S or O;

R = adamantyl-1, bicyclo[6,5,1] heptyl-1 or *tert.*-butyl.

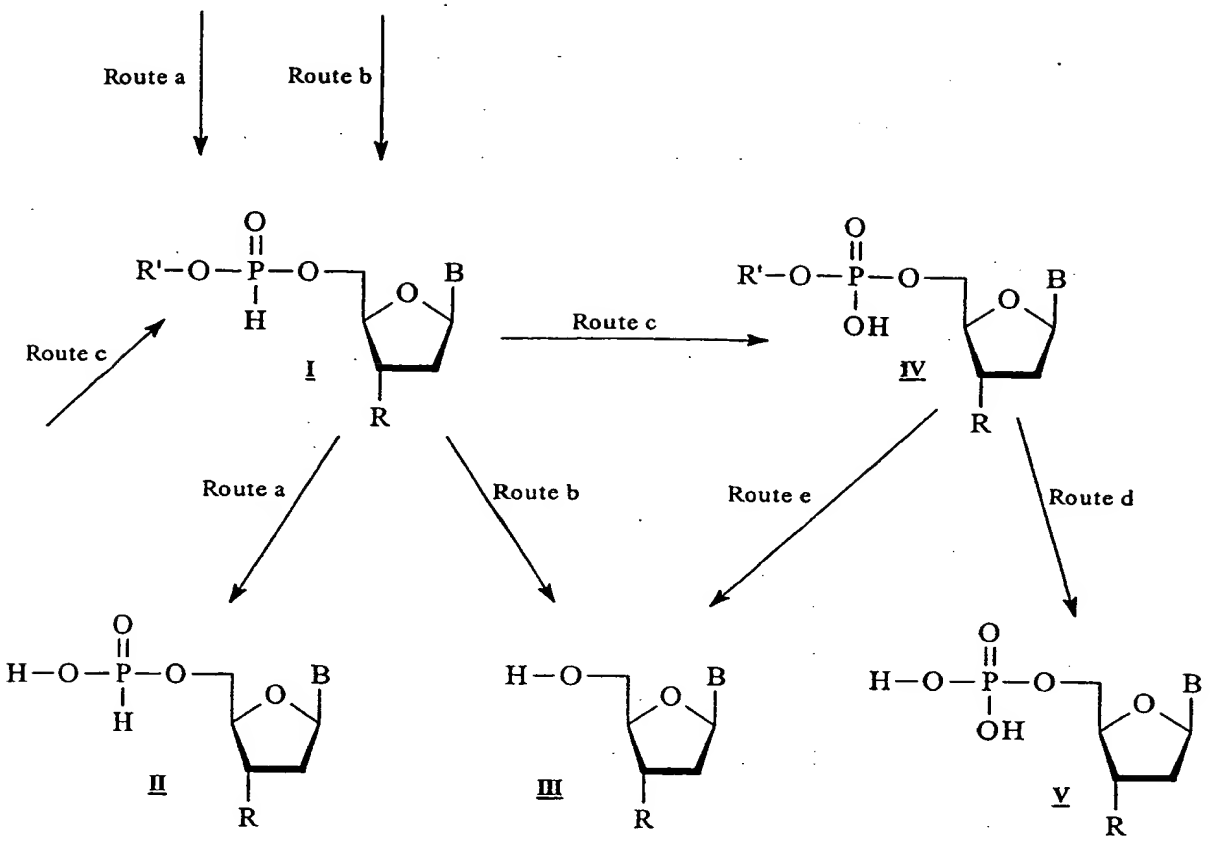

Route a
Route b
Route c
Route c
Route a
Route b
Route e
Route d
I
II
III
IV
V

FIG. 1

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/CA 00/00914

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H19/10 C07H19/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MCGUIGAN, C. ET AL.: "Alkyl hydrogen phosphonate derivatives of the anti-HIV agent AZT may be less toxic than the parent nucleoside analogue" ANTIVIR. CHEM. & CHEMOTHER., vol. 5, no. 4, 1994, pages 271-7, XP002105798 cited in the application the whole document -/--	1-4

[X] Further documents are listed in the continuation of box C.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 December 2000	18/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Bardili, W

2

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INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/CA 00/00914

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H19/10 C07H19/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Bardili, W

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INTERNATIONAL SEARCH REPORT

Inter ›nal Application No
PCT/CA 00/00914

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	CARDONA, V.M.F. ET AL.: "Synthesis and anti-HIV activity of some novel arylphosphonate and H-phosphonate derivatives" ANTIVIR. RES., vol. 42, no. 3, 1999, pages 189-196, XP000944433 cited in the application the whole document	1-4
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INTERNATIONAL SEARCH REPORT

information on patent family members

Inter onal Application No
PCT/CA 00/00914

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		KR 9609930 B	25-07-1996
		WO 8906238 A	13-07-1989